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Characterization of fatty acids, bioactive lipids, and radical scavenging activity of Canterbury bells seed oil

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SUMMARY: The aim of this study was to characterize the chemical composition and radical scavenging activity of Canterbury bells (*Campanula medium*) seed oil. *C. medium* seeds contained 9.2% extractable oil. The lipid classes, fatty acids, phytosterol and tocopherol composition of *C. medium* seed oil were determined. The amount of neutral lipids in the oil was the highest, followed by glycolipids and phospholipids. Linoleic and oleic were the main fatty acids. *C. medium* oil is characterized by high levels of phytosterols and β -sitosterol was the main compound. β -Tocopherol constituted 42.5% of the total tocopherol content followed by γ -tocopherol. The radical scavenging activity (RSA) toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and galvinoxyl radicals of *C. medium* oil were higher than those of extra virgin olive oil. The diverse potential uses of *C. medium* oil may make this plant industrially important.

KEYWORDS: *Campanula medium*; Canterbury bells; Fatty acids; Lipid-soluble bioactives; Radical scavenging potential; Seed oil

RESUMEN: *Caracterización de ácidos grasos, lípidos bioactivos, y actividad de captación de radicales de aceites de semillas de campanillas de Canterbury.* El objetivo de este estudio fue caracterizar la composición química y la actividad de captación de radicales de aceites de semillas de campanillas de Canterbury (*Campanula medium*). Las semillas de *C. medium* contenían 9,2 % de aceite extraíble. Se determinó la composición de las diferentes clases de lípidos, ácidos grasos, fitoesteros y tocoferoles. La cantidad de lípidos neutros en el aceite fue mayoritario, seguido de glicolípidos y fosfolípidos. Linoleico y oleico fueron los ácidos grasos principales. El aceite de *C. medium* se caracteriza por altos niveles de fitoesteros y β -sitosterol fue el compuesto principal. β -tocoferol constituía 42,5 % del contenido total de tocoferol seguido de γ -tocoferol. La actividad de captación de radicales (RSA) a 1,1-difenil-2- picrilhidrazil (DPPH) y radicales galvinoxil de *C. medium* fueron superiores a las de aceite de oliva virgen extra. Los diversos usos potenciales de los aceites de *C. medium* pueden hacer que esta planta pueda ser importante industrialmente.

PALABRAS CLAVE: Aceites de semilla; Ácidos grasos; Bioactivos solubles en lípidos; Campanillas de Canterbury; *Campanula medium*; Poder de captación de radicales

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1. INTRODUCTION

The genus *Campanula* (family Campanulaceae) comprises approximately 300 species. All species are herbaceous, and the name refers to the bell-shaped, blue flowers of the majority of the species. The species are perennials, bi-annuals or annuals (Brandt and Ishimaru, 1998). In addition to their use as ornamental plants, *Campanula* species are used in traditional medicine to treat various diseases such as tonsillitis, laryngitis, bronchitis, and warts. They possess stimulant properties and are used as emetics; they also possess refreshing, antiallergic, antiphlogistic, antioxidant, spasmolytic, antiviral, and antimicrobial properties. Flavonoids are the most important secondary metabolites of the *Campanula* genus (Morton, 1977; Rameau, 1989; Okinaka *et al.*, 2003; Touafek *et al.*, 2011; Yildirim, 2013).

Canterbury bells (*Campanula medium*), also known as the bell flower, is an annual or biennial flowering plant of the genus *Campanula*. The genus Latin name ("campanula"), meaning small bell, refers to the bell-shape of the flower, while the specific epithet ("medium") means that this plant has intermediate characteristics in respect to other species of the same genus. *C. medium* were among the first imported flowers grown in colonial American gardens, where they were valued for their showy three-foot stalks of bell-shaped flowers as well as for their edible roots. The flowering period extends from May to July. The hermaphroditic flowers are either self-fertilized or pollinated by insects (e.g., bees and butterflies). The seeds ripen from August to September and are dispersed by gravity alone (Pignatti, 1982; Brandt and Ishimaru, 1998).

Recent studies on the genus *Campanula* include research on the effect of supplemental light levels and differences in predefined setpoints on the growth development of some *Campanula* species (Kjaera *et al.*, 2012). In another study, three anthocyanins were isolated from acetic acid extract of the light blue-purple flowers of *C. medium*. A new acylated anthocyanin was elucidated to be delphinidin 3-[6-*O*-(α -rhamnopyranosyl)- β -glucopyranoside]-7-*O*-[6-*O*-(4-*O*-(β -glucopyranosyl)-*p*-hydroxybenzoyl)- β -glucopyranoside] as a main anthocyanin, together with two known anthocyanins, violdelphin and delphinidin 3-*O*-rutinoside-7-*O*-glucoside as minor anthocyanins (Toki *et al.*, 2009). Also, the seed coat morphology of 10 *Campanula* species from Turkey was studied using scanning electron microscopy (Akcin, 2009). To the best of our knowledge, no research concerning the chemical composition or antioxidant properties of *C. medium* seeds are yet available.

The trend towards natural ingredients and health promoting products is likely to increase. Non-traditional seeds are being considered because their constituents have unique properties and may

augment the supply of functional products. Owing to their medicinal properties and the health-boosting constituents, non-conventional oils are used in the healthcare industry (Ramadan *et al.*, 2009). Apart from triacylglycerols, crude natural oils contain a number of bioactive lipid compounds with a very diverse chemical makeup. Among the most interesting are the polar lipids, sterols and fat-soluble vitamins. In this investigation, lipid classes, fatty acids, sterols and tocopherols of *C. medium* seed oil have been analyzed for the first time. The objective of this investigation was to obtain an informative profile about the chemical profile of *C. medium* seed oil. The results will be important as an indication of the potentially nutraceutical and economic utility of *C. medium* seeds as a source of edible oils.

2. MATERIALS AND METHODS

2.1. Materials

C. medium seeds were collected in 2012 from the experimental garden at the Faculty of Agriculture, Zagazig University (Egypt). Neutral lipid (NL) standards were from Sigma (St. Louis, MO, USA). The standards used for the identification of glycolipids (GL) were monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebroside (CER), steryl glucoside (SG) and esterified steryl glucoside (ESG), all of which were of plant origin (plant species unknown) and purchased from Biotrend Chemikalien GmbH (Köln, Germany). The standards used for the identification of phospholipids (PL) were phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from bovine liver and phosphatidylcholine (PC) from soybean and were purchased from Sigma. The standards used for the characterization of sterols (ST) were purchased from Supelco (Bellefonte, PA, USA). Lanosterol was purchased from Aldrich (St. Louis, MO, USA). 3 β -hydroxy-5 α -cholestane (dihydrocholesterol) was used as internal standard (ISTD) and was purchased from Sigma. The standards used for vitamin E (α -, β -, γ - and δ -tocopherol) were purchased from Merck (Darmstadt, Germany). Extra virgin olive oil was from a local market (Zagazig, Egypt). The total phenolic compound content in olive oil as determined by the Folin-Ciocalteu at the beginning of the analyses was 337 mg/kg as gallic acid equivalent.

2.2. Methods

2.2.1. Solvent extraction of total lipids (TL)

Seeds (moisture content 12%) were fine powdered using a high-speed mixer, and milled into a fine particle sized (*ca.* 20–50 μ m) meal, then subjected to Soxhlet extraction using *n*-hexane for 14 h. The recovered TL were stored at 4 °C for further analysis.

2.2.2. Column chromatography (CC) and thin-layer Chromatography (TLC) of lipid classes

The TL were separated into different classes by elution with solvents over a glass column (20 mm dia×30 cm) packed with a slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v). NL were eluted with 3 times the column volume of chloroform. The major portion of GL was eluted with 5-times the column volume of acetone and PL with 4 times the column volume of methanol. By means of TLC on Silica gel F₂₅₄ plates (thickness=0.25 mm; Merck, Darmstadt, Germany) a further characterization of GL and PL subclasses was carried out with the following solvent system: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v). For the characterization of NL subclasses TLC plates were developed using *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For the detection of the lipids, TLC plates were sprayed with the following agents: for the marking of all lipids with sulfuric acid (40%), for the marking of GL with α -naphthol/sulfuric acid and for the marking of PL with the molybdate-blue reagent (Kates, 1986; Ramadan *et al.*, 2006). Each spot was identified with lipid standards as well as their reported retention factor (*R_f*) values. Individual bands were visualized under UV light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v).

For the quantitative determination of NL subclasses individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. The data presented are the average of three gravimetric determinations. For the quantitative estimation of GL subclasses, the acetone fraction obtained from CC was separated by TLC in the above given solvent system. The silica gel regions with the corresponding GL subclasses were scraped out followed by a hexose measurement at 485 nm using the phenol/sulfuric acid in acid-hydrolyzed lipids (Ramadan *et al.*, 2006). The percent distribution of each component was obtained from the hexose values. From the extinction values the quantitative amount was determined and related to their portion of the GL fraction. The determined portion was compared with the amount of TL, which had been separated using CC in the main lipid fractions. For the determination of PL, the methanol fraction from CC was also separated using TLC in the above given solvent system. After the scraping out of the individual PL subclasses, classes were brought to react with the hydrazine sulfate/sodium molybdate reagent at 100 °C for 10 min and spectrophotometrically analyzed at 650 nm. From the obtained extinction values *via* a calibration chart for phosphorus the amount of PL was calculated. The individual values were compared to the PL fraction (methanol fraction from CC) and to the amount of TL.

2.2.3. Gas chromatography (GC) analysis of fatty acid methyl esters (FAME)

The fatty acid compositions of TL, NL, GL and PL were determined using GC as described below. Fatty acids were transesterified into FAME using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany) according to Arens *et al.* (1994). FAME were determined on a Shimadzu GC-14A equipped with flame ionization detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas (helium) was 0.6 mL/min and the split value was a ratio of 1:40. A sample of 1 μ L was injected onto a 30 m×0.25 mm×0.2 μ m film thickness Supelco SPTM-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250 °C. The initial column temperature was 100 °C programmed at 5 °C min⁻¹ until 175 °C and held for 10 min at 175 °C, then at 8 °C min⁻¹ until 220 °C and held for 10 min at 220 °C. A comparison was made between the retention times of the samples with those of authentic FAME standard mixture (ME 19, Sigma, St. Louis, MO, USA; 99% purity specific for GC) run on the same column under the same conditions to facilitate identification.

2.2.4. Gas chromatography (GC) analysis of sterols (ST)

The separation of ST was performed after the saponification of oil according to Ramadan *et al.* (2008). TL (250 mg) were refluxed with 5 mL of ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted 3-times with 10 mL of petroleum ether. The extracts were combined and washed 3-times with 10 mL of neutral ethanol/water (1:1, v/v), then dried overnight with anhydrous sodium sulfate. The extract was evaporated in a rotary evaporator at 25 °C under reduced pressure, and then ether was completely evaporated under nitrogen. GC analyses of unsaponifiable residues were carried out using a Mega Series Chromatograph (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with an FID. The following GC conditions were used: DB 5 column (JandW scientific; Falsom, CA, USA) packed with 5% phenylmethylpolysiloxan, 30 m length, 0.25 mm i.d., 1.0 μ m film thickness; carrier gas (helium) flow 0.38 mL min⁻¹. Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μ L. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was <5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

2.2.5. Normal phase high performance liquid chromatography (NP-HPLC) analysis of tocopherols

NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan *et al.* (2010). The analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250×4 mm i.d. LiChrospher-Si 60, 5 µm column (Knauer, Berlin, Germany). The separation of tocopherols was based on isocratic elution when the solvent flow rate was maintained at 1 mL min⁻¹ at a column back-pressure of about 65–70 bar. The solvent system selected for elution was iso-octane/ethyl acetate (96:4, v/v) with detection at 295 nm. Twenty µL of the diluted solution of TL in the mobile phase were directly injected into the HPLC. Tocopherols were identified by comparing their retention times with those of authentic standards. Standard solutions were prepared by serial dilution to a concentration of approximately 5 mg·mL⁻¹ of each tocopherol. Standard solutions were prepared from a stock solution which was stored in the dark at -20 °C. Twenty µL were injected and peak areas were determined to generate standard curve data. All quantification was by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from different concentration levels by linear regression. Based on the chromatographic conditions, repeated injections of different concentrations of the tocopherols were made 3 times in the HPLC system. Injections in triplicate were made at each concentration for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least three times when the variation in any one was routinely less than 5%.

2.2.6. Analysis of phenolic compounds

Aliquots of *C. medium* oil and extra virgin olive oil (2 g) were dissolved in *n*-hexane (5 mL) and mixed with 10 mL methanol-water (80:20, v/v) in a glass tube for two min in a vortex. After centrifugation at 3000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase using a Pasteur pipet and then combined and concentrated *in vacuo* at 30 °C until a syrupy consistency was reached. The lipid residue was re-dissolved in 10 mL methanol-water (80:20, v/v) and the extraction was repeated twice. Hydroalcoholic extracts were re-dissolved in acetonitrile (15 mL) and the mixture was washed three times with *n*-hexane (15 mL each). The purified phenols in acetonitrile were concentrated *in vacuo* at 30 °C and then dissolved in methanol for further analysis. Aliquots of phenolic extracts were

evaporated to dryness under nitrogen. The residue was re-dissolved in 0.2 mL water and diluted (1:30) Folin-Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5% sodium carbonate (0.8 mL) was added. After a further 30 min, the absorbance was measured at 765 nm using UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used for the calibration and the results of triplicate analyses are expressed as parts per million of gallic acid.

2.2.7. Radical scavenging activity (RSA) of *C. medium* oil and extra virgin olive oil toward the DPPH radical

The RSA of *C. medium* oil and olive oil was assayed with DPPH radical previously dissolved in toluene according to Ramadan *et al.* (2009). A toluene solution of DPPH radicals was freshly prepared at a concentration of 10⁻⁴ M. The radical was stable for more than 2 h of kinetic assay in the absence of antioxidant compounds. For evaluation, 10 mg of *C. medium* oil or extra virgin olive oil (in 100 µL toluene) were mixed with 390 µL of the toluene solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1 cm quartz cells after 1, 30 and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of the toluene DPPH solution with or without sample (control) and the inhibition percent was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100$$

2.2.8. RSA of *C. medium* oil and extra virgin olive oil toward the galvinoxyl radical

A Miniscope MS 100 Electron Spin Resonance (ESR) spectrometer (Magnetech GmbH; Berlin, Germany) was used (Ramadan, 2012). The experimental conditions were as follows: measurement at room temperature; microwave power, 6 db; center-field, 3397 G, sweep width 83 G, receiver gain 10 and modulation amplitude 2000 mG. Ten mg of oils (in 100 µL toluene) were allowed to react with 100 µL of a toluene solution of galvinoxyl (0.125 mM). The mixture was stirred in a vortex stirrer for 20 s then transferred to a 50 µL micro pipette (Hirschmann Laborgeräte GmbH, Ederstadt, Germany) and the amount of galvinoxyl radical inhibited was measured exactly 60 s after the addition of the galvinoxyl radical solution. The galvinoxyl signal intensities were evaluated by the peak height of the signals against

a control. Further ESR spectra have been recorded at intervals of 90 s for a total incubation time of 60 min. A quantitative estimation of the radical concentration was obtained by evaluating the decrease in the ESR signals in arbitrary units between 1 and 60 min incubation using a KinetikShow 1.06 Software program (Magnettech GmbH; Berlin, Germany). The reproducibility of the measurements was ± 5 as usual for kinetic parameters.

All experiments were carried out under subdued light conditions. The experiments were repeated at least three times when the variation in any one was routinely less than 5%. All experimental procedures were performed in triplicate and the mean values (\pm standard deviation) were given.

3. RESULTS AND DISCUSSION

For a crop or plant to be suitable for oil production, it must meet the following criteria; (i) the oil content must reach the minimum for commercially valuable exploitation and (ii) the plant must be suitable for high cultivation (Bockisch, 1998). In this study *C. medium* seeds were found to contain about 9.2% crude oil. This confirms that *C. medium* seeds could be considered as a good source of oil.

3.1. Levels of lipid classes and subclasses

The levels of lipid classes and subclasses present in *C. medium* oil and the R_f values of the subclasses are shown in Table 1. Among the TL present in the seeds, the level of NL was the highest (*ca.* 98.7% of TL), followed by GL (0.66% of TL) and PL (0.56% of TL). The subclasses of NL contained triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DAG), esterified sterols (STE) and monoacylglycerol (MAG) in decreasing order. Large amounts of TAG were found (*ca.* 96.9% of

total NL) followed by FFA (*ca.* 1.01% of total NL), while DAG and STE were recovered in smaller amounts. The subclasses of GL were sulphoquinovosyldiacylglycerol (SQD), digalactosyldiglycerides (DGD), cerebrosides (CER), sterylglucosides (SG), monogalactosyldiglycerides (MGD) and esterified sterylglucosides (ESG) as presented in Table 1. SG, ESG, CER and DGD were the prevalent components of GL and comprised more than 90% of the total GL content. The average daily intake of GL in humans was reported to be 140 mg of ESG, 65 mg of SG, 50 mg of CER, 90 mg of MGD and 220 mg of DGD (Sugawara and Miyazawa, 1999). The PL subclasses were separated into four major fractions *via* TLC (Table 1). The TLC fractions revealed that the main PL subclass was PC followed by PE, PI and PS. About half of the total PL was in PC and 28.4% was in PE, while PI accounted for 16.1% of the total PL. PS was measured at lower levels (6.04% of total PL).

3.2. Fatty acid profile of seed oil and its lipid classes

The fatty acid compositions of the TL and lipid classes (NL, GL and PL) are presented in Table 2. Eleven fatty acids were identified in *C. medium* oil, wherein the analysis of FAME gave the proportion of linoleic (C18:2) followed by oleic (C18:1) as the major fatty acids. Both fatty acids accounted for about 83.1% of total FAME. Palmitic acid (C16:0) was the main saturated fatty acid (10.8% of total FAME) followed by stearic acid (C18:0). Trienes including γ -linolenic acid (GLA, C18:3*n*-6) were also detected but in traces. Furthermore, long-chain fatty acids (lignoceric C24:0 and C20:5 EPA) were estimated in traces. *C. medium* oil was characterized by a high level of polyunsaturated fatty acids (PUFA), which accounted for 71.4% of FAME and monounsaturated fatty acids (MUFA) which

TABLE 1. Lipid subclasses ($\text{g}\cdot\text{kg}^{-1}$ TL) in *C. medium* seed oil

Neutral lipid Subclass	R_f values $\times 100^a$	TL $\text{g}\cdot\text{kg}^{-1}$	Glycolipid Subclass	R_f values $\times 100^b$	TL $\text{g}\cdot\text{kg}^{-1}$	Phospholipid Subclass	R_f values $\times 100^b$	TL $\text{g}\cdot\text{kg}^{-1}$
MAG	14	5.67 \pm 0.07	SQD	6	0.40 \pm 0.01	PS	4.7	0.33 \pm 0.01
DAG	39	9.55 \pm 0.11	DGD	17	0.88 \pm 0.02	PI	11	0.88 \pm 0.06
FFA	56	10.3 \pm 0.15	CER	29–35	1.00 \pm 0.05	PC	20	2.70 \pm 0.05
TAG	79	926 \pm 3.30	SG	41	1.97 \pm 0.05	PE	30	1.55 \pm 0.08
STE	95	3.65 \pm 0.05	MGD	64	0.23 \pm 0.03	–	–	–
–	–	–	ESG	76	1.93 \pm 0.06	–	–	–

^aSolvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v).

^bSolvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v).

Results are given as the average of triplicate determinations \pm standard deviation.

Abbreviations: TL, total lipids; MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerols; FFA, free fatty acids; STE, sterol esters; SQD, sulphoquinovosyldiacylglycerol; DGD, digalactosyldiacylglycerol; CER, cerebrosides; SG, steryl glucoside; MGD, monogalactosyldiacylglycerol; ESG, esterified steryl glucoside; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

TABLE 2. Fatty acid profile of *C. medium* oil and its lipid classes

Fatty acid	Total lipids	Neutral lipids	Glycolipids	Phospholipids
		Relative content (% weight percentage)		
C14:0	0.04±0.06	0.03±0.05	0.04±0.05	0.05±0.07
C16:0	10.8±0.55	10.6±0.47	10.9±0.50	11.1±0.46
C16:1	0.29±0.09	0.30±0.08	0.27±0.08	0.28±0.07
C18:0	3.43±0.22	3.36±0.26	3.55±0.24	3.69±0.25
C18:1n-9	13.4±1.04	13.5±1.01	13.4±0.99	13.4±1.02
C18:2n-6	70.9±3.04	71.0±3.05	70.7±3.11	70.3±2.99
C20:0	0.38±0.05	0.35±0.03	0.39±0.09	0.44±0.05
C18:3n-3	ND ^a	ND	ND	ND
C18:3n-6	0.20±0.02	0.26±0.05	0.20±0.03	0.20±0.01
C20:2	0.16±0.02	0.18±0.05	0.14±0.05	0.12±0.03
C22:1n-9	ND	ND	ND	ND
C20:3	ND	ND	ND	ND
C24:0	0.23±0.02	0.20±0.03	0.26±0.04	0.28±0.04
C20:5 EPA	0.17±0.02	0.20±0.04	0.15±0.03	0.13±0.05
C22:6 DHA	ND	ND	ND	ND
Total SFA	14.88	14.54	15.14	15.56
Total MUFA	13.69	13.82	13.67	13.69
Total PUFA	71.43	71.64	71.19	70.75

^aNot detected.

Results are given as the average of triplicate determinations ± standard deviation.

accounted for *ca.* 13.7% of FAME. MUFA have been shown to lower “bad” LDL cholesterol (low density lipoproteins) yet retain “good” HDL cholesterol (high density lipoproteins). This is in fact the major benefit of olive oil over the highly polyunsaturated edible oils, wherein PUFA reduce both the “bad” as well as the “good” serum cholesterol levels in the blood (Ramadan *et al.*, 2010). Fatty acids in NL and polar lipids were not significantly different from each other, wherein linoleic followed by oleic, palmitic and stearic were the main fatty acids. The ratio of unsaturated fatty acids to saturated fatty acids, however, was higher in neutral fractions than in the corresponding polar fractions (GL and PL). Concerning saturated fatty acids (especially palmitic and stearic), GL resembled PL in the higher contents of saturates, while saturated fatty acids were detected in relatively lower levels in the corresponding NL.

From the results, it could be concluded the fatty acid profile of *C. medium* oil shows the lipids as a good source of the nutritionally essential PUFA and oleic acid. Interest in PUFA as health-promoting nutrients has expanded in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Riemersma, 2001). The fatty acid composition and high amounts of

PUFA make the *C. medium* seed oil a special component for nutritional applications. However, as there is no information available about the food use of *C. medium* plant and seeds, the safety of its seed oil shall be investigated before promoting the use of the oil as an ingredient for foods or food supplements.

3.3. Sterol (ST) composition

The Amounts of ST in oils are used for an oil's identification, oil derivatives and for the determination of the oil quality. The concentration of ST has been reported to be little affected by environmental factors or by the cultivation of new breeding lines (Homberg, 1991; Ramadan *et al.*, 2006). *C. medium* oil is characterized by high levels of unsaponifiables (7 g·kg⁻¹ TL). Seven sterol compounds were detected, wherein the sterol marker was β -sitosterol which comprised *ca.* 52% of the total ST content (Table 3). The next major components were Δ^7 -avenasterol and stigmasterol. These three major components accounted for more than 96% of the total ST. Other components, e.g., Δ^5 -avenasterol, lanosterol, Δ^7 -stigmastanol and sitostanol were found in lower levels. Brassicasterol, campesterol and $\Delta^5,24$ -stigmastadinol were not detected in the *C. medium* unsaponifiables. Among the different sterols, sitosterol has been most intensively

investigated with respect to its physiological effects in humans. Many useful and health-promoting effects have been shown for the sitosterol (Yang *et al.*, 2001). Recently, phytosterols have been added to vegetable oils as an example of a successful functional food.

3.4. Tocopherols profile

Tocopherols are the major lipid-soluble, membrane-localized antioxidants in humans and vitamin E deficiency causes defects in the developing nervous system of children and hemolysis in adults. Epidemiologic studies suggest that people with lower vitamin E and other antioxidant intake may be at increased risk for certain types of cancer and atherosclerosis (Gey *et al.*, 1991; Ramadan *et al.*, 2006). Moreover, tocopherols in vegetable oils are believed to protect PUFA from peroxidation. Data about the qualitative and quantitative composition of tocopherols are summarized in Table 3. All tocopherols were present, wherein β -tocopherol constituted 42.5% of the total analytes followed by γ -tocopherol (*ca.* 41.3% of total tocopherols). α - and γ -tocopherols were measured in low levels. α - and γ -tocopherols proved to be the main tocopherols in vegetable oils and fats. γ -tocopherol occurred in high concentrations in camelina, linseed, cold-pressed rapeseed and corn oil (Schwarz *et al.*, 2008). α -tocopherol is the most efficient antioxidant of tocopherols, while β -tocopherol has 25–50% of the antioxidative activity of α -tocopherol, and

γ -isomer 10–35% (Kallio *et al.*, 2002). Despite agreement that α -tocopherol is the most efficient antioxidant *in vivo*, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness *in vitro*, especially when compared to γ -tocopherol (Kamal-Eldin and Appelqvist, 1996). Levels of tocopherols detected in *C. medium* oil may contribute to the stability of the oil toward oxidation.

3.5. RSA of *C. medium* oil in comparison with extra virgin olive oil

Interest has increased in the free radical theory of the cause of disease, particularly in vascular diseases and cancer. These developments have led to the investigation on the role of antioxidants in disease protection. A free radical is defined as any chemical species that has one or more unpaired electron. Antioxidants quench those radicals and if the supply of antioxidants is inadequate, a chain reaction takes place that may lead to damaged tissue. Previous study on the radical scavenging properties of vegetable oils had used different solvents to dissolve the oils and the free radicals. Thus, the results were difficult to compare because the reactions occurred under different conditions. In contrast, a simple experiment (Ramadan *et al.*, 2010) has been performed using the same solvent (toluene) to dissolve the oil samples and the free radicals.

TABLE 3. Levels of sterols and tocopherols in *C. medium* seed oil

Compound	mg·kg ⁻¹	%
Brassicasterol	Nd ^a	0.0
Campesterol	110±0.09	2.5
Stigmasterol	500±0.08	10.9
Lanosterol	170±0.02	3.6
β -Sitosterol	2410±0.33	52.0
Δ 5-Avenasterol	490±0.09	10.6
Sitostanol	390±0.07	8.3
Δ 5, 24-Stigmastadinol	Nd	0.0
Δ 7-Stigmastenol	Nd	0.0
Δ 7-Avenasterol	560±0.23	12.1
α -Tocopherol	180±0.06	15.9
β -Tocopherol	491±0.09	42.5
γ -Tocopherol	477±0.11	41.3
δ -Tocopherol	3.0±0.001	0.3

^a Not detected.

Results are given as the average of triplicate determinations ± standard deviation.

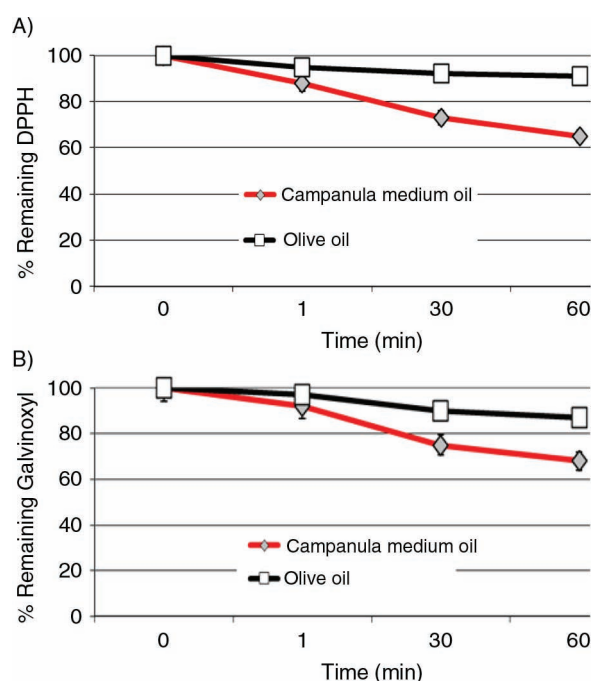


FIGURE 1. Antiradical effect of *C. medium* seed oil and extra virgin olive oil on DPPH radicals (A) as measured by changes in absorbance values at 515 nm and on galvinoxyl radicals (B) as recorded by ESR.

This allowed us to characterize and compare the RSA of all samples under the same conditions.

Apart from the fatty acid profile, the stability of vegetable oils depends on the presence of minor fat-soluble bioactives and the initial amount of hydroperoxides. RSA of the *C. medium* oil and extra virgin olive oil (as a standard crude oil with respective high levels of nutritive antioxidants and bioactives) were compared using stable DPPH and galvinoxyl radicals. Figure 1 shows that *C. medium* oil had higher RSA values than olive oil. After 60 min incubation, 35% of DPPH radicals were quenched by *C. medium* oil, while olive oil was able to quench 9% (Figure 1A). ESR measurements showed the same pattern, wherein *C. medium* oil quenched 32% galvinoxyl radicals and olive oil quenched 13% after 60 min of reaction (Figure 1B).

C. medium oil and olive oil have different patterns of fatty acid and lipid-soluble bioactives. *C. medium* oil was characterized by higher phenolic content (466 mg·kg⁻¹) than extra virgin olive oil (337 mg·kg⁻¹). It could be said that the RSA of oils can be interpreted as the combined action of different endogenous antioxidants. However, when polar fractions (mainly polar lipids and phenolics) are found in high levels, strong RSA of these components can be expected (Ramadan *et al.*, 2010). The stronger antiradical action of *C. medium* oil compared to olive oil may be due to (i) the differences in levels and composition of polar lipids and unsaponifiables (phytosterols and tocopherols) (ii) the diversity in structural characteristics of potential antioxidants present, (iii) a synergism of polar lipids with other components present, and (iv) different kinetic behaviors of potential antioxidants.

4. CONCLUSIONS

Knowledge concerning the composition and properties of *C. medium* seeds would assist in efforts for industrial, nutritional and medical application of this plant. It is difficult to find data about the bioactive lipids and antioxidant compounds of *C. medium* seed oil in the literature. It could be concluded that *C. medium* seeds give considerable oil yield and the oil seems to be a good source of essential fatty acids and lipid-soluble bioactives. The high linoleic and oleic acid content makes the oil nutritionally valuable. Tocopherols and sterols at the level estimated may be of nutritional importance in the application of the seed oil. *C. medium* seeds could be nutritionally considered as a new non-conventional supply for pharmaceutical industries and edible purposes.

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